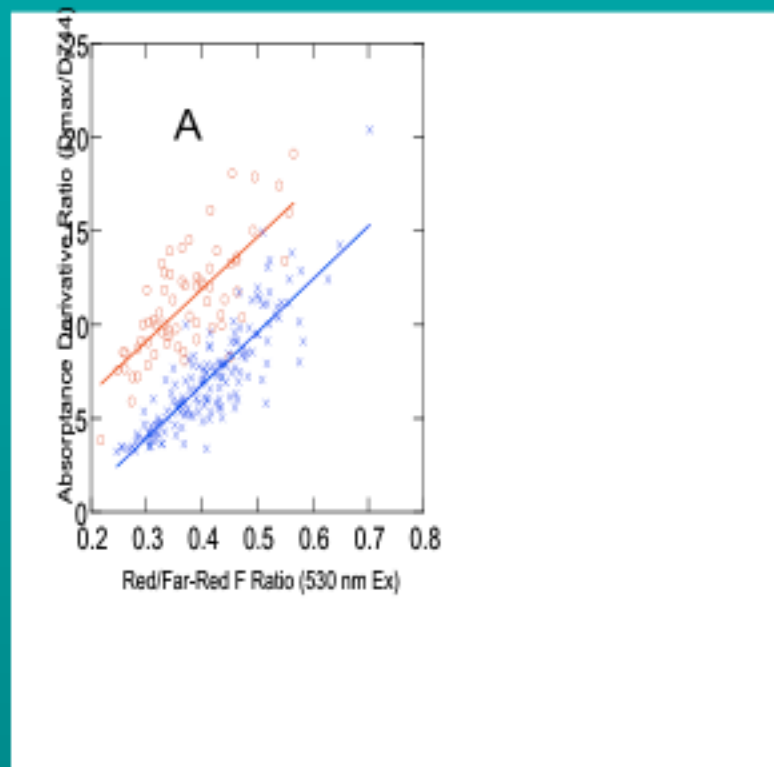
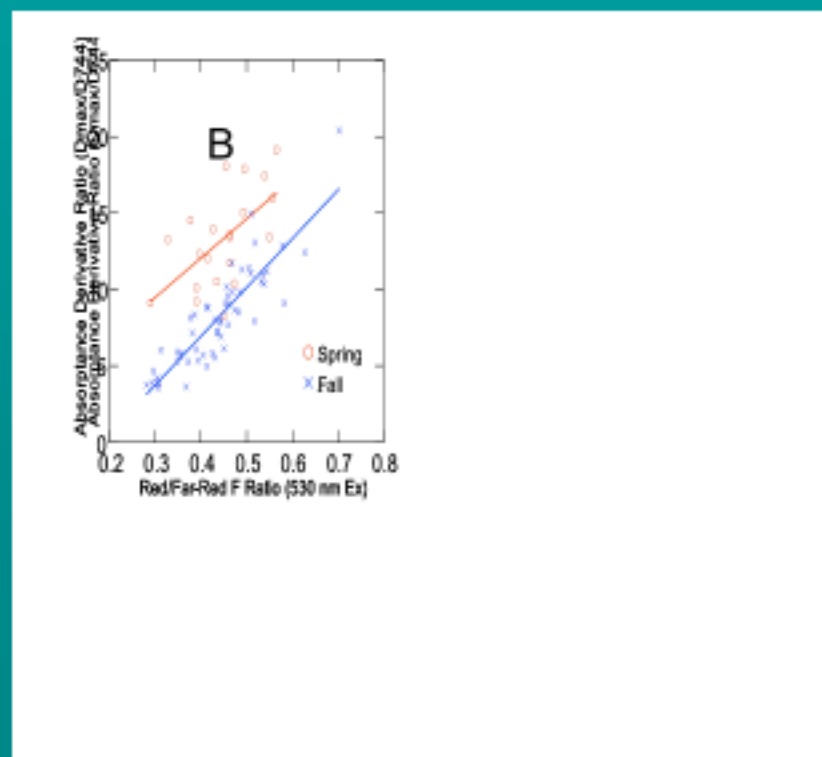




Relationship between the R / FR F ratio and Dmax/D744



A. Positive linear relationship between the red/far-red fluorescence ratio resulting from excitation at 530 nm and the absorbance red-edge derivative ratio Dmax/D744 ($r = 0.875$, $p < 0.001$, $n = 219$). 2002 and 2003 data and all three species combined.



B. Positive linear relationship between the red/far-red fluorescence ratio resulting from excitation at 530 nm and the absorbance red-edge derivative ratio Dmax/D744 ($r = 0.877$, $p < 0.001$, $n = 76$). 2002 and 2003 data for red maple only.



Theme # 6

Measurements Supporting Instrumentation Development



Future Prospects for Laser Induced Fluorescence Imaging



- Spectra-Physics Frequency-Tripled Nd:YAG Laser (355 nm)
- Gated 12-bit 2 Stage Peltier Forced Air Cooled CCD Camera
- Multispectral Image Acquisition

The Laser Induced Fluorescence Imaging System (LIFIS) simultaneously captures four fluorescence emission bands for each pulse of the laser. A fast gated image intensifier enables operation under both laboratory and field conditions without the interference of ambient light.

LIFIS was custom fabricated by NASA to characterize spatial variability in fluorescence over a wide range of vegetation scenes. Fluorescence emissions can be captured from vegetation *in vivo* without disturbing, moving, or excising plant material from its natural environment.



LIFIS Multi-Spectral Imaging

(10 nm FWHM)



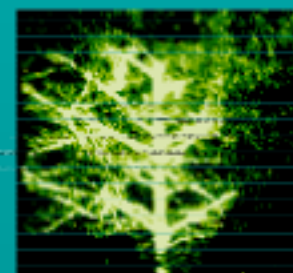
Native riparian zone species: Sweet gum (*Liquidambar styraciflua* L.)



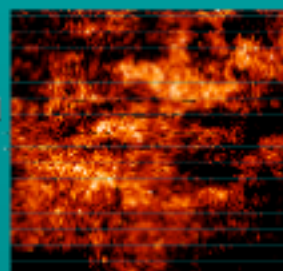
Blue Band
(450 nm)



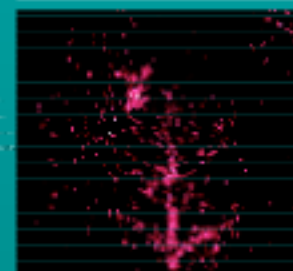
Green Band
(530 nm)



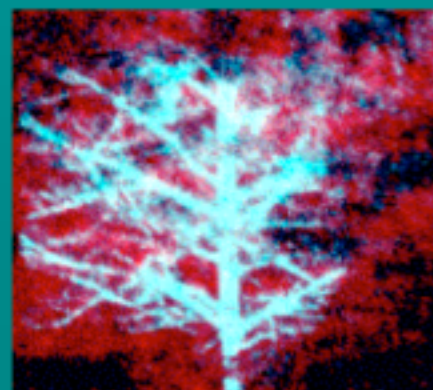
Far-Red Band
(730 nm)



Red Band
(680 nm)



Classification algorithms and ratio imagery are useful in isolating fluorescence emissions from plant components of scientific interest.



RGB Composite
R: Far-Red Band
G: Green Band
B: Blue Band

Target Area 9m²



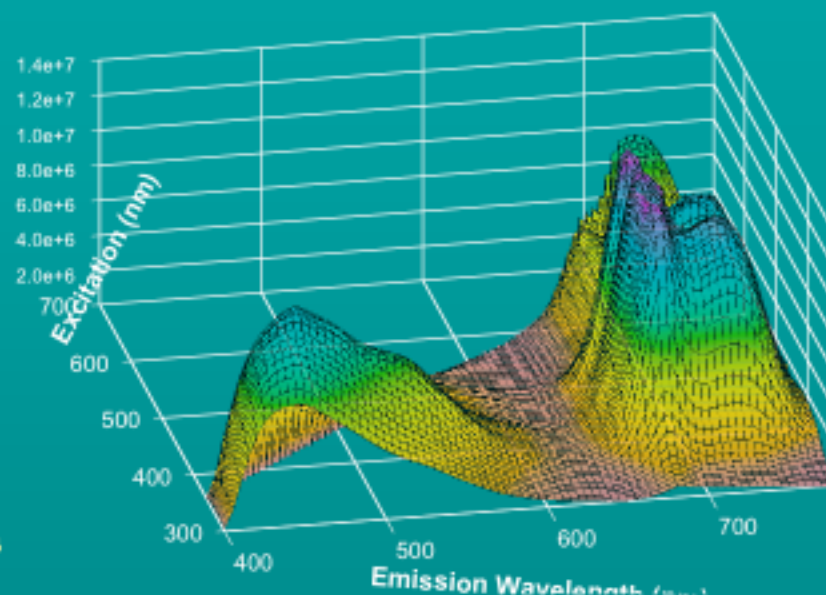
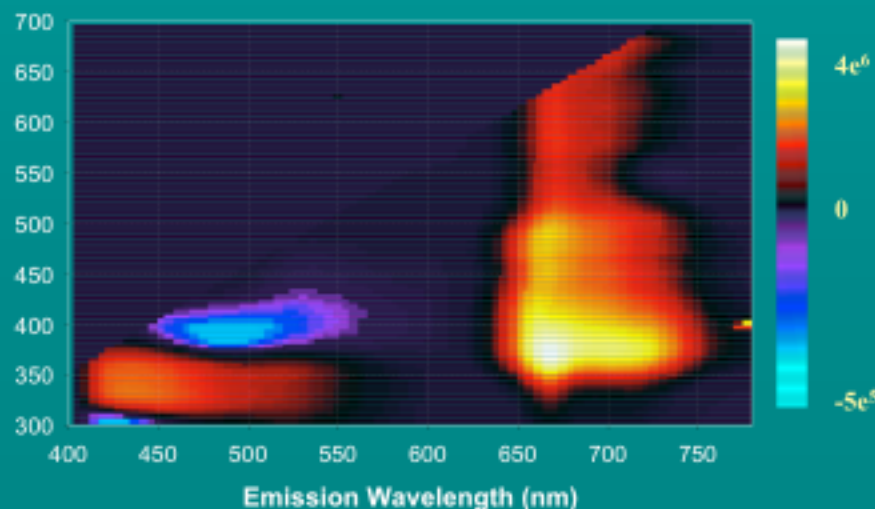
Changes in Fluorescence Associated with Vegetation Stress



Fluorescence Excitation and Emission Matrix (EEM) *Zea mays* L.

The fluorescence emission from vegetation varies as a function of excitation wavelength. The EEM allows one to observe unique spectral features associated with plant stress.

Difference EEM High – Low N



Difference EEM dictates that there are several discrete regions where significant N induced changes in the primary plant emission bands can be observed.



Fluorescence EEM Maxima



The fluorescence EEM provides a complete spectral characterization of light emissions and can provide vital statistics, such as, mean wavelengths for fluorescence excitation (Ex) by emission (Em) maximum across several plant species.

| Fluorescence Band | | Corn | Soybean | Poplar | Gum | Maple | Vegetation Average |
|-------------------|----|----------|----------|---------|----------|----------|--------------------|
| Blue | Ex | 352* | 346 ± 43 | 341 ± 5 | 353 ± 29 | 342 ± 8 | 347 ± 25 |
| | Em | 443 ± 12 | 460 ± 6 | 453 ± 5 | 446 ± 11 | 443 ± 14 | 450 ± 11 |
| Green | Ex | - | 400 ± 8 | 419* | 409 ± 9 | 418* | 410 ± 10 |
| | Em | - | 529 ± 7 | 538 ± 5 | 540 ± 11 | 538* | 536 ± 8 |
| Red #1 | Ex | 423 ± 12 | 425 ± 10 | 437* | 431 ± 10 | 436* | 430 ± 10 |
| | Em | 681* | 688 ± 6 | 686* | 691 ± 6 | 688* | 687 ± 5 |
| Red #2 | Ex | 482 ± 13 | 473 ± 7 | 471 ± 8 | 467* | 467* | 472 ± 8 |
| | Em | 681* | 694 ± 7 | 690* | 698 ± 5 | 693 ± 5 | 691 ± 7 |
| Far-Red #1 | Ex | 424 ± 14 | 424 ± 10 | 437* | 432 ± 11 | 437* | 430 ± 10 |
| | Em | 734* | 740* | 740* | 740* | 740* | 739* |
| Far-Red #2 | Ex | 479 ± 14 | 471 ± 8 | 471 ± 8 | 467* | 467* | 470 ± 8 |
| | Em | 735* | 740* | 740* | 740* | 743* | 740* |
| Far-Red #3 | Ex | 663 ± 15 | 662 ± 10 | 669* | 669* | 668* | 665 ± 8 |
| | Em | 736* | 740* | 740* | 740* | 740* | 739* |

* Standard deviation is less than the data acquisition interval of 5 nm (n=20).

Corp, L.A., Middleton, E.M., McMurtrey, J.E., Campbell, P.K.E., Butcher, L.M., "Fluorescence Sensing Systems: Optimizing Sensitivity to Vegetation Parameters", *Applied Optics*, submitted 4/2004.



Suggested Developments for Laser Induced Fluorescence



- Incorporate the frequency doubled 532 nm Nd:YAG laser excitation with UV EX to enhance the Chl F signal and enable a series of dual excitation fluorescence ratios. Combine with Red and Far-Red emission or Reflectance Bands → observe Chl F.
- Continue to incorporate advances in fixed emission solid-state and tunable lasers to further the capability multi-excitation fluorescence imaging for plant stress detection and remote sensing implementation.



Theme # 7

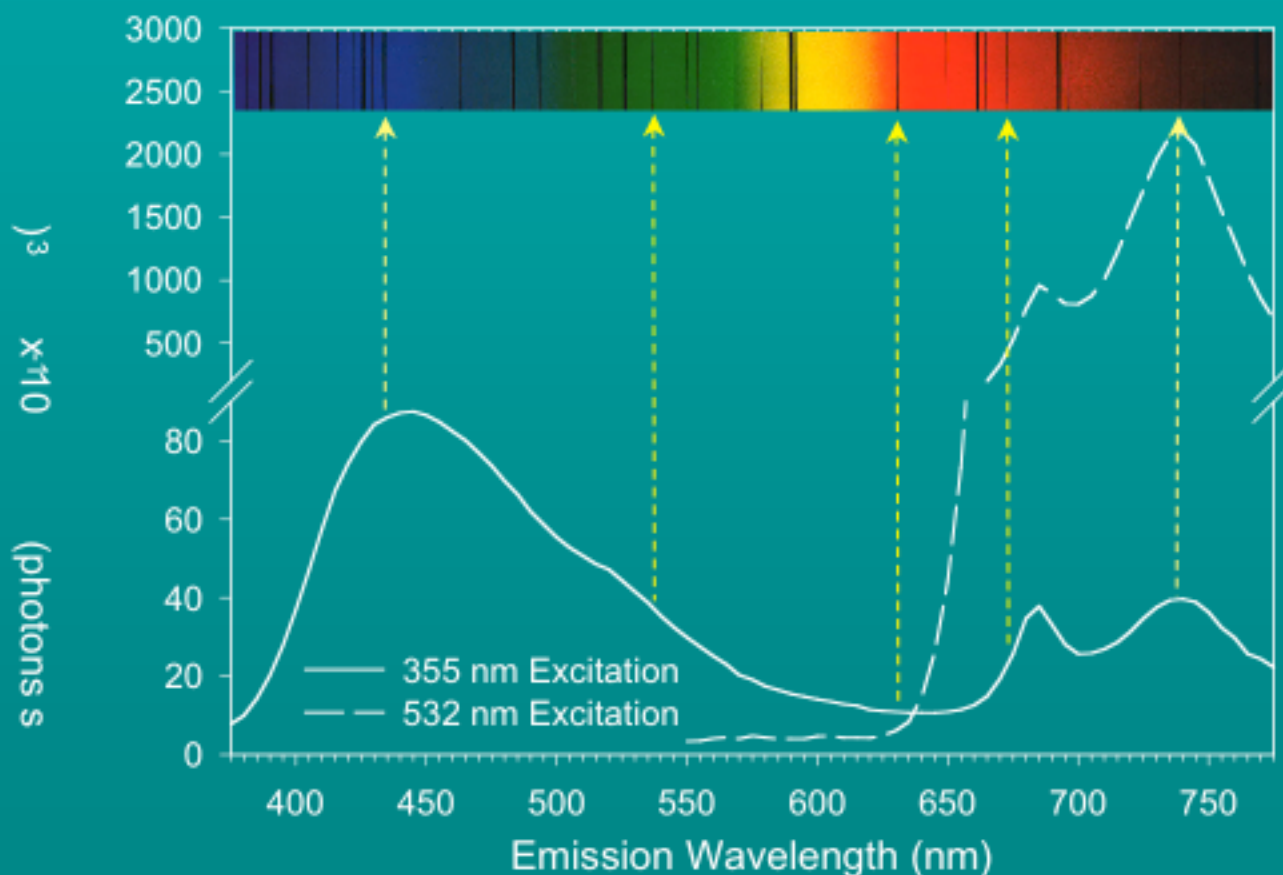
Relating Active Fluorescence to Passive Fluorescence Observations



Prospects for Passive Fluorescence Sensors



The Fraunhofer line-depth method measures the fluorescence fill in of narrow gaps in the solar emission spectrum





Prospects for Passive Fluorescence Sensors



- Accuracy of carbon and primary productivity models would clearly be enhanced by merging reflectance and fluorescence characteristics.
- The Fraunhofer line-depth method makes it possible to isolate chlorophyll fluorescence from reflected sunlight.
- Proposals have been made to develop remote sensing interferometer technology to discriminate solar induced fluorescence in selected Fraunhofer lines. Combined efforts of NASA centers (GSFC, LRC, SSC, USDA, UNH, & Michigan Aerospace Corp.) are pointing to the development of interferometer technology for diagnosing carbon dynamics in vegetation.



Our Recent Project Accomplishments



We have shown:

- 1] F & R spectral indicators for N application rates and foliar C/N content.
- 2] F & R spectral responses to environmental stresses.
- 3] F & R spectral indicators for photosynthetic function.
- 4] ChlF contributes to “red edge” reflectance.
- 5] R/FR ChlF ratio varies with EX wavelength.
- 6] R/RF ChlF ratio from 530EX resembles “red edge” reflectance ratios, and may have application to other NASA laser/lidar instrumentation.
- 7] A red edge derivative ratio is related to C/N content.
- 8] Accessory pigments can be estimated from ChlF via EX spectra.
- 9] UV EX produces unique F information.